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Cell Walls and the Convergent Evolution of the Viral Envelope

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SUMMARY

Why some viruses are enveloped while others lack an outer lipid bilayer is a major question in viral evolution but one that has received relatively little attention. The viral envelope serves several functions, including protecting the RNA or DNA molecule(s), evading recognition by the immune system, and facilitating virus entry. Despite these commonalities, viral envelopes come in a wide variety of shapes and configurations. The evolution of the viral envelope is made more puzzling by the fact that nonenveloped viruses are able to infect a diverse range of hosts across the tree of life. We reviewed the entry, transmission, and exit pathways of all (101) viral families on the 2013 International Committee on Taxonomy of Viruses (ICTV) list. By doing this, we revealed a strong association between the lack of a viral envelope and the presence of a cell wall in the hosts these viruses infect. We were able to propose a new hypothesis for the existence of enveloped and nonenveloped viruses, in which the latter represent an adaptation to cells surrounded by a cell wall, while the former are an adaptation to animal cells where cell walls are absent. In particular, cell walls inhibit viral entry and exit, as well as viral transport within an organism, all of which are critical waypoints for successful infection and spread. Finally, we discuss how this new model for the origin of the viral envelope impacts our overall understanding of virus evolution.

INTRODUCTION

The majority of organisms that act as hosts for viruses possess a cell wall. Cell walls are robust layers that surround the cell membrane and are best known in plants, fungi, protists, algae, and bacteria. Cell walls are clearly ancient, and while the similarity of cell wall components indicates a shared ancestry among algae and plants (1), studies of brown algae and Archeaplastida (i.e., green and red algae and land plants) suggest that cell walls have evolved convergently (2). The cell wall has a variety of functions from protection to the maintenance of cell shape, although its most important role is to provide structural support to counteract high internal osmotic pressure. The cell wall is also a selective filter, allowing free diffusion of small molecules and ions. Experiments with cell walls in plants and bacteria have determined an exclusion size of approximately 50 to 60 kDa (3–5). This allows the diffusion of important signaling molecules, such as phytohormones in plants, but not virus particles.

Cell walls differ in number and composition, depending on the organism. Several plants have a secondary cell wall (6), while bacteria and *Archaea* possess only a single cell wall. The diversity of cell wall components has led to several classification systems based on their complexity and composition, such as the classification systems for algae (7) and flagellates (8), and these systems can be used to assess the rigidity of a cell wall. While the majority of bacteria possess a rigid cell wall due to the presence of peptidoglycan, in some cases, such as *Mycoplasma*, there is no such rigid "shell," and the cell walls consist of a plasma membrane reinforced with glycocalyx, a glycoprotein polysaccharide (9, 10). Similarly, most members of the *Archaea* domain have a crystalline protein layer, called the surface layer (S-layer), as their cell wall lacks peptidoglycans (10–12). As a consequence, the cell walls of most *Archaea* are less rigid than those of bacteria.

In marked contrast, animal cells lack cell walls and are surrounded by a flexible lipid bilayer, the cell membrane, that can contain numerous important functional modifications such as receptors or other membrane-bound structures. These structures

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are responsible for molecule uptake and excretion, are involved in cell signaling, and maintain a stable osmotic pressure and pH (13). Hence, the cell walls found in plants, fungi, protists, algae, and bacteria provide a rigid and strong barrier for viral entry and exit not seen in animal cells. Critically, viruses cannot enter cells that possess cell walls by endocytosis or exit these cells by budding, and instead they rely on a number of different approaches.

While viral genomes encode the structural proteins they require, enveloped viruses acquire a major component of their envelope from the host cell through budding and are able to modify it by inserting their own proteins (14). The envelope may be acquired from the host cell membrane or intracellular compartment, such as the endoplasmic reticulum or Golgi compartment (15). Upon virus entry, each layer of a virus serves to overcome a specific host cell barrier. After each successful breach, the corresponding layer of the virus is lost, eventually delivering the unpacked genomic payload to its origin of replication. Inversely, successful virus exit involves the acquisition of these layers. However, the pathways for virus entry and exit differ substantially, especially among viruses infecting cells surrounded by a cell wall.

To understand the evolution of the viral envelope, we reviewed and compared the mechanisms of virus entry, spread, and exit among all known virus families. Strikingly, this revealed that enveloped viruses predominantly infect organisms without cell walls, while viruses without an envelope can infect hosts with and without cell wells, although the majority of their hosts possess cell walls. From this analysis, we hypothesize that the lack of an envelope is a specific viral adaptation to the presence of cell walls, while the viral envelope is an adaptation to hosts that lack cell walls. Although there are a number of exceptions to this simple evolutionary rule, closer inspection reveals that these individual adaptations support the general distinction noted above. Indeed, we show that viruses from organisms possessing cell walls have evolved a variety of ways to ensure successful infection and spread. While entry pathways of known viruses have been compared and analyzed extensively in previous publications (16–21), this is, to our knowledge, the first synthesis that links viral evolution to the structure of host cells.

VIRUS ENTRY, TRANSMISSION, AND EXIT

We selected 101 virus families from the 2013 release of the International Committee on Taxonomy of Viruses (ICTV) (22). We excluded the viroid families *Avsunviroidae* and *Pospiviroidae*, virus satellites, and the family *Metaviridae*, since they contain eukaryotic retrotransposons. Of the 101 virus families analyzed, 65 were nonenveloped virus families, while 37 were enveloped (the *Iridoviridae* can be both enveloped and nonenveloped and hence were included in both groups [23, 24]). To identify the host range of these virus families, we created seven broad classes of host organisms based on their identified hosts (see Data Sets S1 and S2 in the supplemental material) and their taxonomic position in the tree of life (D. R. Maddison and K.-S. Schultz, Tree of Life Web Project [http://tolweb.org]). In total, we identified 123 host types, of which 64 were animal cells with no cell walls, while 59 had cells surrounded by a cell wall. All bacteria were grouped in the class (simplified taxonomic class) "Eubacteria" and hence distinct from the *Archaea*. The eukaryotes were split into five classes (simplified taxonomic classes): "Plants" (which contains all plants and algae), "Protozoa," "Fungi," "Invertebrates," and "Vertebrates" (Fig. 1). "Fungi" contains all *Eumycota*, while animals were subdivided into "Vertebrates" (*Chordata*) and "Invertebrates" (all non-*Chordata*). The remaining members of the animal clade were classified as "Protozoa." Importantly, this classification was developed only as a general guide for data analysis and did not impact any of the major conclusions drawn.

We then analyzed the 101 virus families to determine the taxonomic distribution of the presence/absence of envelopes among viruses. This revealed a strong association between the presence of the viral envelope and the absence of a cell wall in the host organism. Specifically, the 65 nonenveloped virus families infected 79 host types, of which 49 had cells with a cell wall while 30 did not (Table 1 and Fig. 1). In contrast, of the 37 enveloped virus families, only 10 infected host types with cell walls compared to 34 host types without cell walls. Hence, the majority of host types with cell walls are infected by nonenveloped viruses, while the majority of enveloped viruses infect animal cells. Only a few enveloped viruses are known to infect cells with cell walls, representing unique cases that are likely to be highly specialized adaptations (see below).

We also analyzed the pathways for virus entry, transmission, and exit (Tables 2 and 3; see below). Viral entry into animal cells relies on endocytosis pathways for both enveloped and nonenveloped viruses. However, endocytosis is not possible in organisms that possess a cell wall, since it creates an important physical barrier. Virus release by excretion pathways or budding is similarly hindered. Of the 65 nonenveloped virus families analyzed, 21 are released by lysis, while 10 are released in a nonlytic pathway (Table 3). In contrast, only five enveloped virus families exit the host cell by lysis, while 21 utilize a nonlytic pathway, mostly budding or the endosomal sorting complex required for transport (ESCRT). ESCRT is a conserved molecular complex that modulates membrane scission into the cytoplasm. However, several viruses have managed to use parts of the ESCRT complex for budding and subsequent release into the cytoplasm (26). In addition, some plant and fungal viruses spread vertically, never leaving the cell (16). Finally, our analysis of pathways of viral transmission within hosts showed that, among multicellular organisms with cell walls like plants, the capsid or ribonucleoprotein (RNP) is the key factor, such that an envelope is not required (see below). Accordingly, we propose that nonenveloped viruses are an adaptation to the evolution of the cell wall, while the viral envelope constitutes an adaptation to cells without cell walls (i.e., animal cells). We now discuss, in more detail, how these observations relate to aspects of the virus life cycle.

Although our review of the literature covers all those virus families for which data are available—entry and exit pathways for 71 and 57 virus families, respectively—it is important to note that it does not include all known viruses (Tables 2 and 3). Although we are able to describe pathways from all known host kingdoms, most data are necessarily from the better-known viruses. Clearly, it will be important to determine whether the generalities noted here can be extended to all known virus groups, including those only recently described, and it is striking that there is relatively little data from most archaeal and insect viruses.

Virus Entry

The major role of membranes in animal cells is to create distinct compartments and to receive and send signals from outside the cell. Therefore, viruses have to enter and exit animal cells in a systemic infection or to reach their target tissue. Viruses have

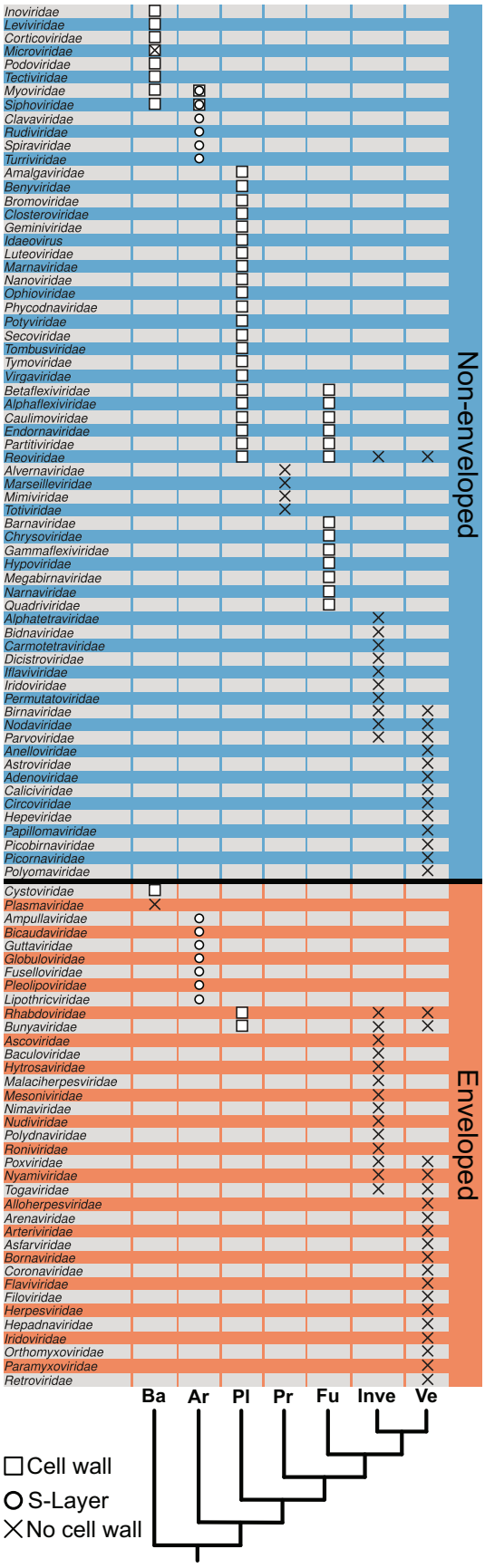

TABLE 1 Summary of the pattern of association between virus envelopes (presence or absence) and hosts (with and without cell wall) among 101 virus families[a]

Host	No. of virus families		
	Nonenveloped	Enveloped	Total
With cell wall	49	10	59
No cell wall	30	34	64
Total	79	44	

[a] The S-layer of *Archaea* has been treated as cell wall. Note that some virus families can infect hosts with and without cell wall and are therefore present in more than one category.

overcome this barrier in animals by hijacking endo- and exocytosis pathways.

Animal viruses have evolved several ways to enter animal cells, although these pathways are always based on the flexibility of the cell membrane (17). This flexibility allows different pathways for virus uptake for both enveloped and nonenveloped viruses. Viruses are adapted to endocytosis pathways, as they offer entry points usually used for nonspecific uptake of fluids, solutes, or particles. As an example, vaccinia virus enters cells by mimicking an apoptotic body, thereby triggering macropinocytosis (27, 28). Virus uptake through endocytosis is induced upon binding of the virus to cell surface receptors (20). For enveloped viruses, uptake into animal cells involves the fusion and subsequent release of the capsid (29), while nonenveloped viruses can create pores in the cell membrane to deliver their viral genome (30, 31). A single virus can induce several endocytosis pathways as observed for dengue virus and HIV-1. While both can enter cells by triggering macropinocytosis (32, 33), additional entry pathways for dengue via the clathrin-mediated pathway (34) and HIV-1 through fusion have been observed (35).

Such entry pathways are blocked in plants and bacteria due to the presence of the cell wall. While the plant cell wall allows diffusion of water and ions, the diffusion of macromolecules is restricted. However, endocytosis-like pathways have been observed in plants (36) and bacteria (37). Lonhienne et al. (37) used green fluorescent protein (GFP) to highlight endocytosis in *Gemmata obscuriglobus*, a budding bacterium with Gram-negative cell wall structure (38), and showed that GFP was able to diffuse through the cell wall. The maximum exclusion size for cell walls of plants and bacteria is approximately 60 kDa (3–5). We estimated the diameter of a spherical protein that can diffuse freely through the cell wall to be ~5.126 nm, which approximately corresponds to the width of two DNA double helices (Appendix). Consequently, while the GFP, with a molecular mass of 26.9 kDa and a diameter of 2.4 nm (39, 40), is able to diffuse through cell walls, viruses cannot. Critically, therefore, the intrinsic rigidity of cell walls in plants means that plant pathogens have evolved a variety of ways to penetrate and infect their hosts (41). We now discuss some of these adaptations.

FIG 1 Association between known virus families and the presence of a cell wall, surface layer (S-layer), or absence in the hosts they infect. The schematic phylogenetic tree represents our simplified taxonomic classes as defined in the text. The abbreviations for the different host classes are as follows: Ba, Bacteria; Ar, Archaea; Pl, Plants; Pr, Protozoa; Fu, Fungi; Inve, Invertebrates; Ve, Vertebrates.

TABLE 2 Cell entry pathways of the virus families analyzed[a]

Cell entry pathway	Virus family [reference(s)][b]
Endocytosis	*Caliciviridae* (102)
	Hepeviridae (111)
	Parvoviridae (123, 124)
	Phycodnaviridae (46)
	*Hepadnaviridae** (135)
Macropinocytosis	*Adenoviridae* (103)
	Birnaviridae (108)
	Papillomaviridae (117)
	Mimiviridae (125)
	Totiviridae (130)
	*Filoviridae** (136–138)
	*Herpesviridae** (141)
	*Nodaviridae** (145)
	*Paramyxoviridae** (148, 149)
	*Poxviridae** (27)
Clathrin mediated	*Adenoviridae* (104–106)
	Astroviridae (112)
	Circoviridae (118)
	Luteoviridae (126)
	Papillomaviridae (131, 132)
	Pestiviridae (139)
	Picornaviridae (142, 143)
	Polyomaviridae (146)
	Reoviridae (150, 151)
	Iridoviridae(*) (152)
	*Coronaviridae** (154)
	*Arenaviridae** (156)
	*Arteriviridae** (158–160)
	*Asfarviridae** (162)
	*Baculoviridae** (166, 167)
	*Bornaviridae** (169)
	*Bunyaviridae** (170)
	*Filoviridae** (171)
	*Flaviviridae** (172, 173)
	*Orthomyxoviridae** (174)
	*Paramyxoviridae** (175)
	*Retroviridae** (176, 177)
	*Rhabdoviridae** (178)
	*Togaviridae** (179–181)
Caveolae	*Papillomaviridae* (107)
	Picornaviridae (113)
	Polyomaviridae (119, 120)
	*Hepadnaviridae** (127)
	*Retroviridae** (133)
Lipid raft	*Birnaviridae* (108)
	Caliciviridae (114)
	*Orthomyxoviridae** (128)
Fusion	*Corticoviridae* (109)
	Phycodnaviridae (45)
	Picornaviridae (121)
	Tectiviridae (109)
	Iridoviridae(*) (134)
	*Arenaviridae** (140)
	*Baculoviridae** (144)
	*Coronaviridae** (147)
	*Cystoviridae** (50)
	*Herpesviridae** (153)
	*Malacoherpesviridae** (155)
	*Paramyxoviridae** (157)
	*Plasmaviridae** (59, 161)
	*Polydnaviridae** (163–165)
	*Retroviridae** (168)
Ejection[c]	*Microviridae* (47)
	Myoviridae (115, 116)
	Podoviridae (122)
	Siphoviridae (129)
Pilus retraction	*Inoviridae* (61)
	Leviviridae? (43)
Membrane penetration	*Picobirnaviridae*? (110)

[a] Families where no entry pathways have been published are not listed.
[b] Enveloped virus families are indicated by a * symbol, while (*) indicates virus families containing enveloped and nonenveloped forms. A? symbol indicates putative exit pathways. The corresponding source publication(s) or reference(s) is shown in parentheses at the end of an entry.
[c] Ejection indicates membrane penetration, cell wall digestion, and genome ejection.

In plants and fungi, viruses do not actively breach the cell wall. Plant viruses are obligate intracellular parasites in that they remain with their host indefinitely but can be transmitted by vectors, fungi (42), mechanical injuries, or vertically (16). Fungal viruses have adapted to cell walls by using hyphal anastomosis (fusion of encountering vegetative hyphae) for horizontal transmission and a persistent lifestyle for vertical transmission. Vertical transmission allows fungal viruses to stay in the host (43). Similarly, some plant viruses remain asymptomatic inside the host, relying in vertical transmission through seeds (16, 21, 44).

The situation is complex in algae. While algae share similarities with plants with respect to cell architecture, notable exceptions exist. *Chlorella*, a single-cell green algae, is infected by *Paramecium bursaria* chlorella virus 1 (PBCV-1) (45). PBCV-1 has an internal membrane (that is, the membrane is surrounded by the capsid). To enter its host, PBCV-1 degrades the *Chlorella* cell wall and fuses its inner membrane with the cell membrane (45). Another algal virus, *Emiliana huxleyi* virus 86, belongs to the *Coccolithovirus* genus and infects a wide range of eukaryotic algae in marine and freshwater environments. *Emiliana huxleyi* is a marine calcifying unicellular phytoplankton. Rather than a typical cell wall, these phytoplanktons possess a characteristic calcite covering that surrounds the cell membrane. Although it belongs to the *Phycodnaviridae* family, like PBCV-1, *Emiliana huxleyi* virus 86 has an additional outer membrane that covers the capsid, and to infect its host, the virus fuses its outer membrane with the host membrane or enters via an endocytic process (46). Since budding of *Emiliana huxleyi* virus 86 particles from infected *Emiliana huxleyi* has been demonstrated (46), we assume that the cell covering is not tight enough to exclude viral particles. However, it has been proposed that the calcified shell offers a certain degree of viral defense (46). It should be noted that its capsid may possess cell wall-degrading enzymes, although they are not required in this case. This example of an "animal virus-like" entry mechanism shows that viruses infecting unicellular algae have evolved several approaches to enter their hosts.

Similar to plant pathogens, most bacteriophage have evolved

TABLE 3 Cell exit pathways of the virus families analyzed[a]

Cell exit pathway	Virus family [reference(s)][b]
Unknown/nonlytic[c]	*Hepeviridae* (182, 183) *Inoviridae* (61) *Luteoviridae* (189) *Mesoniviridae* (194) *Nodaviridae* (198) *Papillomaviridae* (202) *Rudiviridae* (207) *Totiviridae* (212) *Bornaviridae** (216) *Bunyaviridae** (221) *Fuselloviridae** (225) *Malacoherpesviridae** (155)
ESCRT	*Picornaviridae* (93) *Arenaviridae** (186) *Filoviridae** (190) *Flaviviridae** (195) *Rhabdoviridae** (199) *Hepadnaviridae** (203) *Herpesviridae** (208) *Paramyxoviridae** (213) *Poxviridae** (217) *Retroviridae** (222)
Budding	*Phycodnaviridae* (46) *Reoviridae* (187) *Asfarviridae** (191) *Baculoviridae** (144, 196) *Coronaviridae** (200) *Iridoviridae*(*) (204) *Nyamiviridae** (209) *Orthomyxoviridae** (214) *Plasmaviridae** (218, 219) *Togaviridae** (223)
Lysis	*Annelloviridae* (184, 185) *Astroviridae* (188) *Birnaviridae* (192, 193) *Caliciviridae* (197) *Corticoviridae* (201) *Leviviridae* (205, 206) *Marnaviridae* (210, 211) *Marseilleviridae* (215) *Microviridae* (220) *Mimiviridae* (224) *Myoviridae* (226) *Parvoviridae* (227) *Phycodnaviridae* (228) *Picornaviridae* (229) *Podoviridae* (230, 231) *Polyomaviridae* (232) *Reoviridae* (233) *Rudiviridae* (234) *Siphoviridae* (82, 235) *Tectiviridae* (236) *Turriviridae* (237) *Adenoviridae** (238) *Ascoviridae**? (239) *Circoviridae** (240, 241) *Cystoviridae** (242) *Polydnaviridae**? (243)

[a] Virus families without (published) exit pathways are not listed.
[b] Enveloped virus families are indicated by a * symbol, while (*) indicates families containing enveloped and nonenveloped forms. A? symbol indicates putative exit pathways. The corresponding source publication(s) or reference(s) is shown in parentheses.
[c] Unknown/nonlytic indicates release pathways where no lytic pathway exists but viral release has been observed.

diverse entry pathways (Fig. 2). All known bacteriophage use lytic enzymes to penetrate the cell wall, while different mechanisms have been described to overcome the bacterial membranes. Most bacteriophage follow a three-step program: (i) puncture the outer cell wall, if present; (ii) digest the cell wall; (iii) insert the phage genome into the host cell. Tail-less, nonenveloped bacteriophage, such as ϕX174, form a tube to deliver their genome into the host (47). However, enveloped bacteriophage have also been observed. Interestingly, these envelopes can surround the capsid, as in the case of *Cystovirus*, or the envelope can be encapsulated by a capsid, as in the case of *Corticovirus* or *Tectivirus* (48). To infect Gram-negative bacteria, enveloped bacteriophage found in the *Cystoviridae* and *Corticoviridae* families fuse their envelope with the outer membrane of their hosts (48–50). Phages PRD1 and Bam35 belong to the *Tectiviridae*. Both are nonenveloped, but the capsid encloses an internal membrane containing the genome. Despite their similarity, PRD1 infects Gram-negative bacteria, while Bam35 infects Gram-positive bacteria. The entry pathway from Bam35 differs in some steps form PRD1 (Fig. 2). Both phages form a tube for DNA delivery which is initiated by capping vertices from the capsid. The osmotic difference between the capsid and cytosol pushes the internal membrane through a special vertex in the capsid. The emerging membrane has lytic properties and digests the cell wall, thereby forming a tube for subsequent DNA delivery (51, 52). PRD1 possess proteins that are loosely associated with the internal membrane and are able to puncture the outer membrane (53, 54). In contrast, as Bam35 infects Gram-positive bacteria, it does not need to perforate an outer membrane, although the genes for outer membrane perforation are present in its genome (55). In addition, these phages differ in how they pass the internal membrane (56–58). Bam35 depolarizes the internal membrane, while PRD1 does not, although mechanisms by which it functions are not fully understood. Bacteriophage infecting Gram-positive bacteria do not need to pass an outer membrane and can attack the cell wall directly. In the case of bacteriophage that have an envelope covered by a protein capsid, such as Bam35, the envelope facilitates the fusion with the inner membrane (57). Notably, *Plasmavirus*, an enveloped bacteriophage, exclusively infects *Mycoplasma*, one of the few bacteria without a cell wall (59).

Another bacteriophage family has evolved a very different approach. Members of the *Inoviridae* attach to the pili of Gram-negative bacteria (60). The retraction of the pili brings the capsid into contact with the inner membrane where it disassembles and is released into the cytoplasm (61). This approach circumvents the outer membrane and cell wall altogether, abolishing the need for an envelope and cell wall-digesting properties (Fig. 2).

The host range for enveloped bacteriophage does not include Gram-positive bacteria, since the envelope cannot fuse and the cell wall is not digested, as in the case of *Cystovirus*. The *Inoviridae* similarly do not possess an envelope, since it is not required for infection, as they bypass the outer membrane and cell wall by using the pili of their host. The presence of the cell wall requires cell wall-degrading enzymes for successful infection, which are largely associated with base plates and capsids of bacteriophages.

Overall, the analysis of viral entry pathways strongly supports our hypothesis that the presence of a virus envelope is associated with the absence of cell walls and vice versa, such that these two traits have an intimate evolutionary relationship (Fig. 1). In particular, the presence or absence of a viral envelope is clearly better

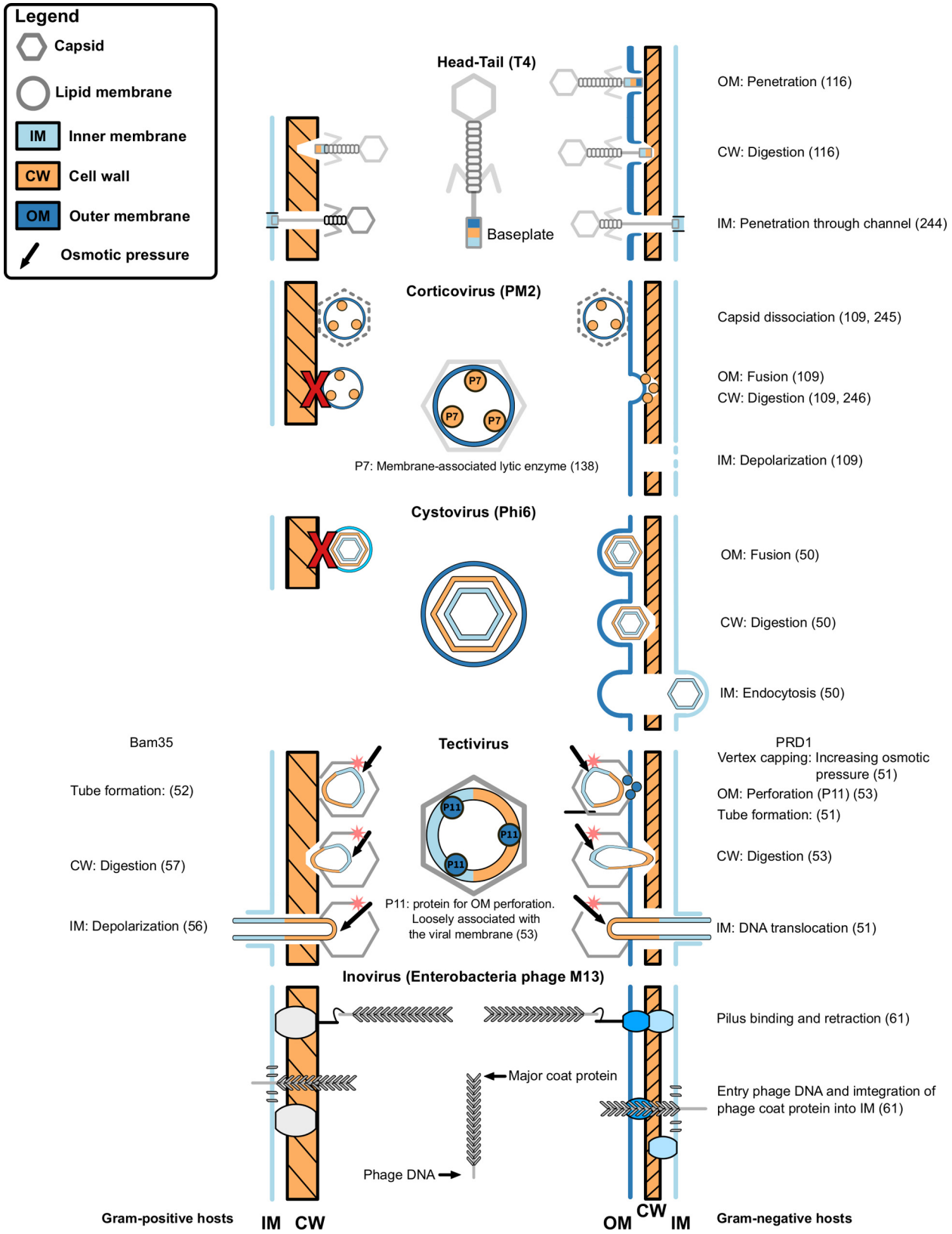

FIG 2 Schematic overview of different bacteriophage entry mechanisms. Several different entry mechanism for nonenveloped (Head-Tail, Corticovirus, and Tectivirus), enveloped (Cystovirus), and filamentous (Enterobacteria phage M13) bacteriophage are shown. Structures are not drawn to scale, and only key structures for viral entry are shown and color coded according to the part they breach during entry, e.g., components responsible for cell wall degradation have the same color as the cell wall indicated in the legend. Associated membrane proteins are indicated as circles. Mechanisms for Gram-positive bacterial hosts are shown on the left, while those Gram-negative hosts are shown on the right. Numbers in parentheses indicate references for the corresponding step (steps without references are putative and inferred by the authors) (see references 50 to 53, 56, 57, 61, 109, 116, and 244 to 246). No Gram-positive hosts are known for corticovirus and cystovirus, and a red X indicates possible interference of the cell wall onto the entry mechanism.

associated with cell structure, especially the presence or absence of a cell wall, than to a specific type of host species.

Intrahost Virus Spread

We now examine how the presence of the cell wall, which influences cell-to-cell communications, impacts viral spread within an individual host. Once plant viruses enter epidermal or mesophyll cells, systemic transport is possible by taking advantage of the plant cell architecture. It is known that plant viruses move from cell to cell by plasmodesmata and across whole vascular plants by phloem (62). Multicellular fungi are either coenocytic (large cells with several nuclei) or the cells are separated by septa, i.e., end walls that can be perforated and therefore connect neighboring hyphae. The movement of viral capsids within or between fungi is not restricted and can occur horizontally by hyphal anastomosis, a naturally occurring process in which two hyphal cells create a fusion aperture to allow the migration and exchange of nuclei and cytoplasm (63, 64).

Due to a general inability to infect new hosts by penetrating the cell walls, plant and fungal viruses rely on different mechanisms to gain entry into new hosts, with arthropod vectors a key element. Using vectors to infect new hosts is possible, since the cell wall is breached upon feeding, which we therefore propose to be a secondary adaptation in plant and fungal viruses (see below). Viruses in insects can be classified into two groups based on their mode of transmission—noncirculative and circulative (18)—which reflect how long a virus is viable in the vector during transmission to a new host. Noncirculative transmission is essential for viruses that remain within the vector at the mouthparts or foregut and need to be immediately inoculated into a new host after acquisition by the vector (65). In contrast, circulative transmission allows longer times between acquisition and transmission of the virus into the new host by circulating across the gut, hemolymph, and salivary gland before being inoculated into a new host. Circulative plant and insect viruses can undergo this process with or without replication.

Transport across the plasmodesmata requires a virus-encoded movement protein which interacts with the plasmodesmata to allow the passage of the virus particles (66). The transport of viruses within plants occurs either as a RNP or viral capsid (67, 68) but, importantly, not as enveloped viruses. Experiments in tomatoes infected with *Tomato leaf curly virus* (69, 70) and *Tomato bushy stunt virus* (TBSV) (71–73) showed that viruses without the ability to form capsids were transported from cell to cell but with a lower efficiency. Interestingly, only four plant-infecting virus genera possess an envelope: *Cytorhabdovirus*, *Nucleorhabdovirus* (both of which are members of the family *Rhabdoviridae*), *Emaravirus*, and *Tospovirus*. Since the envelope is not required for cell entry and subsequent cell-to-cell movement, we argue that its limited presence in these genera is because it facilitates vector-borne viral transmission.

Rhadboviruses are unusual in that they are able to infect both plants and animals, with *Cytorhabdovirus* and *Nucleorhabdovirus* able to bud in the plant and insect host (74). In plants, budding virions are found in the perinuclear space and at the cell membrane (74). Since the enveloped form of plant viruses is not transported to neighboring cells (67, 75), it has to be assumed that enveloped *Rhabdoviridae* in plants are transmitted solely by vectors. This scenario has also been reported for *Tospovirus*, the only genus of the *Bunyaviridae* infecting plants. Mature *Tosposvirus* virions accumulate in the plant cells, waiting to be transmitted by feeding thrips (68). The enveloped, vector-borne emaviruses have been recently discovered in several plant species (76), and their capability for cell-to-cell movement is likely based on the capsid, rather than the envelope (77).

In the enveloped *Tospovirus*, two transmembrane glycoproteins G_N and G_C, are required for vector transmission, as repeated passages through plants led to accumulated mutations in those proteins that subsequently impaired insect transmission (78). In addition, targeted point mutations in G_N and G_C inhibited transmission through thrips (79), although plant infection was not impaired. *Cytorhabdovirus* and *Toposvirus* are all circulative and persistent within the vector. In addition, Rhabdoviruses show a wider array of vectors, while *Toposvirus* is associated only with thrips (18, 80). This strongly suggests that the envelopes of enveloped plant viruses are an adaptation to the vector, not the host.

Cell walls impair cell-to-cell communications, and structures like the plasmodesmata serve as communication channels between plant cells. Viruses have adapted them for viral movement within the plant hosts. While plant viruses can acquire an envelope in plant cells, the envelope is not required for viral cell-to-cell movement, which is facilitated by the capsid or RNP. That all enveloped plant viruses are vector-borne strengthens our theory that nonenveloped viruses are an adaptation to the cell wall, and envelopes are needed only upon vector-aided translocation due to the fact that viral transport is possible as capsid, RNP, or naked DNA/RNA, such that the viral envelope is not required.

Virus Exit

The absence of a cell wall in animal cells favors endocytosis for cell entry and budding for cell exit. Budding pathways have been successfully adopted by viruses. Several enveloped viruses hijack the ESCRT pathway (19, 81) that is responsible for a variety of functions in a cell, including endosomal sorting, receptor signaling, and cytokinesis (26). Only a few enveloped viruses lyse the host cell to be released, while virtually all nonenveloped viruses exit the host cell through lysis (Table 2). Interestingly, nonenveloped viruses infecting animals do not use excretion pathways and lyse their host cell (Table 2).

With the exception of the *Inoviridae*, all bacteriophage escape the host cell through lysis. *Inoviridae* encode three proteins that create a secretion channel through the cell wall and bacterial membranes (61). Recent research with Gram-negative bacteria indicates that both the cell wall and outer membrane are actively disrupted through a spanin complex (82). Permeabilization of the inner membrane is the first step, whereby holins and pinholins, small viral membrane proteins, are secreted into the inner membrane of the host and upon activation allow cell wall-degrading enzymes to leave the cytoplasm (83–87). The subsequent release of endolysins into the periplasm degrades the peptidoglycan. While the spanin complexes are required to disrupt the outer membrane, its mechanics are unknown (88). Similarly, the release pathway of the enveloped bacteriophage *Cystovirus* is currently unclear. Bacteriophage that do not possess an envelope can induce lysis by holins without being permeabilized themselves. In contrast, virus envelopes can be targeted by holins, especially as the envelope is acquired from the host.

Lysis of a bacterial cell involves membrane-disrupting proteins. Therefore, viruses that acquire an envelope from the inner membrane of the host turn themselves into a putative target for mem-

brane permeabilization. This, in turn, would release capsids that are capable of digesting cell walls but not getting past the outer or inner membranes of bacteria. Hence, we propose that members of the *Tectiviridae* and *Corticoviridae* evolved the outer capsid to protect their envelope during host cell lysis. Since virus particles cannot diffuse through the cell wall, exocytosis pathways in plants and bacteria are not used for viral release.

EVOLUTIONARY IMPACT OF CELL WALLS ON VIRAL ENVELOPES

Our association study of 101 viral families and their hosts revealed a strong relationship between enveloped viruses and animal host cells and nonenveloped viruses and host cells with cell walls. An extensive literature review of viral entry, transmission, and exit strategies of these viral families supports our main hypothesis that cell walls were central to the evolution of nonenveloped viruses, while the lack of a cell wall provides an adaptive advantage to viruses with envelopes. The cell wall constitutes an important physical barrier that cannot be breached by endocytosis for entry or exocytosis for exit. In bacteria, where membranes are present, viral envelopes are used to get past either the outer or inner membrane but lack the sophisticated arsenal of receptors found on enveloped viruses that infect animal cells.

The Viral Envelope Is a Result of Convergent Evolution

A variety of models can be proposed to explain the evolution of the viral envelope. If we assume that early viruses were enveloped, then they must have lost their envelope several times (Fig. 3A). Conversely, if early viruses were not enveloped, as seems more likely, then they have gained their envelope several times (Fig. 3B). A third possibility is the initial coexistence of enveloped and nonenveloped viruses and subsequent selection in the corresponding hosts leading to either gain or loss of the envelope (Fig. 3C). The scattered presence of envelopes among viral taxa strongly suggests that they have evolved convergently, which we propose reflects the presence or absence of cell walls in phylogenetically diverse host species.

It is also possible that host jumps allowed nonenveloped viruses to infect animals and enveloped viruses to infect hosts with cell walls. For example, a large number of new RNA viruses have recently been identified in arthropods, constituting a potentially huge viral reservoir (89). Since arthropods have a close ecological relationship to both plants and vertebrates, host jumps from plants to animals via arthropods are not unlikely. As mentioned above, animal cells show less discrimination between enveloped and nonenveloped viruses than organisms that possess a cell wall, and the ability of plant virus capsids to release genes into mammalian cells has been demonstrated (89). Hence, the pivotal position of arthropods between plants and vertebrates could have facilitated the adaptation of nonenveloped viruses to vertebrates.

The only enveloped viruses in plants are *Emaravirus*, *Bunyavirus*, and *Rhabdovirus*. As noted above, the envelopes of plant viruses appear to be an adaptation to the vector, rather than to the plant, and hence could be the result of a host jump. Since all other plant viruses are not enveloped, they have obviously lost the envelope or were never enveloped. However, the former scenario seems highly unlikely, since plants evolved before insects (90, 91). Entering the plant through mechanical injuries after being transported by environmental factors like wind or rain would still be possible, although likely inefficient. As a consequence, early enveloped plant viruses appear to have few ways to be transmitted.

Plant viruses can move within their host by plasmodesmata and phloem, while fungal viruses can transverse their hosts due to perforated septa. These specialized cell-to-cell links evolved to facilitate cell communication, overcoming the rigidness and impermeability of cell walls. Crucially, we argue that this development also led to preferential infection by nonenveloped viruses. Hence, most plant and fungal viruses are not enveloped, since fusion or budding from a plant or fungal cell is not feasible due to the presence of a cell wall and because transport inside the host is possible only via the RNP or capsid. The adaptation of viral capsids or RNPs for transport by plasmodesmata and the later emergence of arthropods means that early plant viruses were very likely nonenveloped. In turn, this means that *Emaravirus*, bunyaviruses, and rhabdoviruses infected plants subsequent to the emergence of arthropods.

Cystoviridae and *Plasmaviridae* are the only known enveloped bacteriophage families, and both have a very limited known host range, the former infecting only *Pseudomonas*, while the latter infect only *Mycoplasma*, suggesting that the envelope is a highly specialized adaptation. Although several bacteriophage with internal membranes exist, such membranes lack the receptors required for cell entry. Therefore, viruses infecting cells with a cell wall do not need an envelope *per se*, and if it is present, it serves as a tool to gain access to the cell wall by fusion with an outer membrane or fusion with the inner membrane after cell wall digestion. As mentioned earlier, numerous bacteriophage encode their own membrane proteins but gain the lipids required for their membrane from their hosts. Therefore, a scenario of coexisting nonenveloped and enveloped early viruses (Fig. 3C) is unlikely. Assuming early bacteriophage were able to synthesize their own lipids and lost this ability over time in favor of using host lipids, we speculate that bacteriophage will have a wider host range than currently seen, as in the case of the cystoviruses where a mutation in a coding region would allow them to infect Gram-positive hosts (Fig. 2).

In sum, we argue that early viruses were likely nonenveloped with the viral envelope a later adaptation (Fig. 3A). In support of this, nonenveloped bacteriophage show the simplest adaptation for bacterial infection, since they are able to enter and exit their hosts with the least interference. In contrast, enveloped bacteriophage need to deal with the lytic pathway and limited entry possibilities. Without a cell wall, endocytosis of enveloped and nonenveloped viruses would most likely occur, as seen in animal viruses. However, the cell wall renders endocytosis and exocytosis not feasible. The use of lytic enzymes to exit the host requires the permeabilization of the cell membrane, thereby potentially threatening the virus itself. Without an envelope, membrane permeabilization is not a concern. This, in turn, influences virus entry, since membranes are required for several bacteriophage to enter the host cell.

The enormous diversity among virus families greatly complicates phylogenetic analysis, including whether virus envelopes have been gained or lost through evolutionary history. However, previous studies have revealed clear evolutionary relationships between the so-called alphavirus-like (nonenveloped) and flavivirus-like (enveloped) positive-sense RNA viruses (92) and among the *Mononegavirales* group of negative-sense RNA viruses (89). In addition, it has also been shown that nonenveloped picornavi-

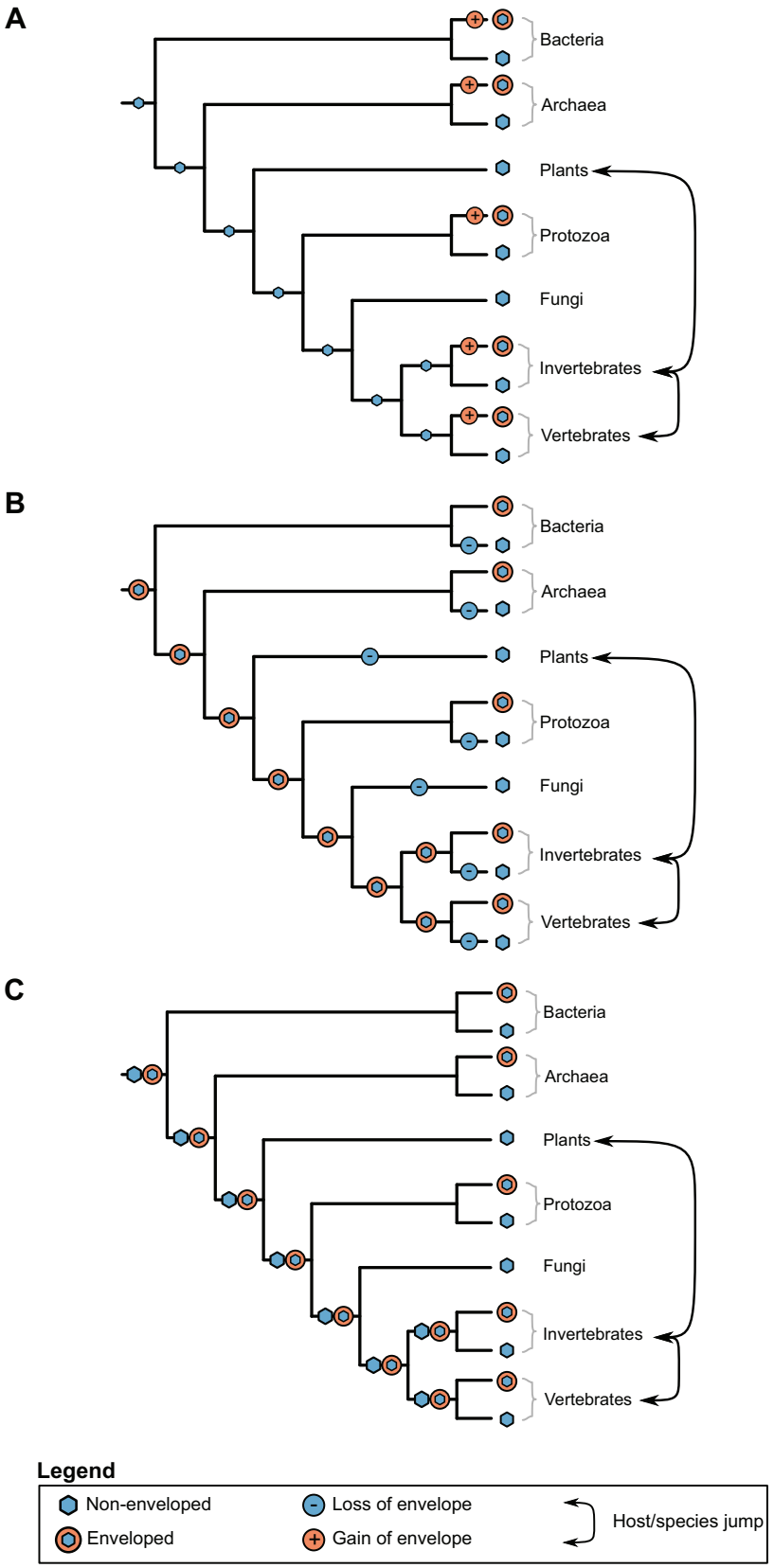

FIG 3 Three models for the loss and/or gain of the viral envelope during evolutionary history, as well as putative host jump events. The phylogenetic tree is the same as that used in Fig. 1. (A) Early, nonenveloped viruses with subsequent gain (multiple times independently) of the viral envelope. (B) Early, enveloped viruses with its subsequent loss in multiple host lineages. (C) Early, coexisting nonenveloped and enveloped viruses.

ruses can acquire an envelope from the cellular membrane (93). Together, these data offer support to the idea that the viral envelope evolved convergently.

The Viral Envelope as an Adaptation to Animal Cells

Entering animal cells requires the correct signals to trigger endocytosis. Animal cells use membrane-bound receptors for cell signaling, which viruses use to gain entry into the cell. The viral envelope is advantageous in such cases, since different viral receptors can be expressed, providing the virus with the ability to trigger more than one endocytosis pathway. In contrast, capsids (in the absence of envelopes) offer less flexibility to attach different receptors. Acquiring the host's membrane not only offers less visibility to the immune system but allows a flexible way to mount receptors. For example, Ebola virus uses glycoproteins to mask its epitopes, a strategy not applicable to viral capsids due to its rigidity. Experiments with the nonenveloped plant viruses *Luteovirus* and *Begomovirus* revealed that they interact with GroEL, a chaperone of a symbiotic bacterium in aphid vectors (94, 95). This interaction is required for circulative transmission and protects against degradation in the vector (96, 97). Chaperones are not only involved in protein folding but also in membrane translocation. *Luteovirus* and *Begomovirus* enter the primary salivary glands in the vector via endocytosis before infecting the host via the saliva. We assume that GroEL functions as an envelope substitute, since the receptors on the viral capsids do not trigger endocytosis, indicating that capsids have a limited flexibility to attach different receptors. However, cases where nonenveloped viruses can attach to several receptors are also known. For example, foot-and-mouth-disease virus is known to attach to two different receptors *in vivo*, integrin (98) and heparan sulfate proteoglycans (99).

A common denominator among organisms with cell walls is the lack of an adaptive immune system. While innate immunity recognizes pathogens in a generic way, the adaptive immune system has virtually unlimited possibilities to recognize pathogens. Viral membranes offer the possibility to adapt to different cell types by expressing or including different varieties of membrane-bound entry receptors than on a single capsid. Such complexity is not required to evade innate immune systems. In addition, viral transport from the entry site to different organs increases the exposure of the viruses to the adaptive immune system. In such a scenario, the envelope may serve as a decoy, as the virus appears to be a host cell.

In sum, our extensive review has revealed a close association between cell walls and nonenveloped viruses that was not bound to particular types of host organism. The cell wall provides a physical barrier that hinders the interaction of receptors on the viral envelope with receptors in the cell membrane, an interaction that is central to the infection of animal cells. Although there are exceptions to this important evolutionary generality, we show that they can be considered to be individual adaptations. We also propose that early viruses were nonenveloped and that the viral envelope has evolved several times independently, reflecting the diversity of hosts encountered; this provides a new perspective on our understanding of virus origins and evolution.

APPENDIX

Calculating the Radius of a Spherical Protein of 60 kDa To Estimate the Particle Exclusion Size for Cell Walls

We calculated the volume of the protein (V) and used this to calculate its diameter. The average density of a protein of 60 kDa can be calculated as described previously (100, 101), resulting in 1.4114 g/cm^3. The volume for a protein of this size is then calculated as follows:

$$V(\mathrm{nm}^3) = \left\{[1/p(\mathrm{g/cm}^3) \times 10^{21}(\mathrm{nm}^3/\mathrm{cm}^3)]/\mathrm{Na(Da/g)}\right\} \times M(\mathrm{Da}) \quad (1)$$

$$V(\mathrm{nm}^3) = \left\{[0.70851(\mathrm{cm}^3/\mathrm{g}) \times 10^{21}(\mathrm{nm}^3/\mathrm{cm}^3)]/\mathrm{Na(Da/g)}\right\} \times M(\mathrm{Da}) \quad (2)$$

$$V(\mathrm{nm}^3) = [7.08516(\mathrm{nm}^3/\mathrm{g})/\mathrm{Na(Da/g)}] \times M(\mathrm{Da}) \quad (3)$$

$$V(\mathrm{nm}^3) = 0.00117\ (\mathrm{nm}^3/\mathrm{Da}) \times M(\mathrm{Da}) \quad (4)$$

$$V(\mathrm{nm}^3) = 0.00117(\mathrm{nm}^3/\mathrm{Da}) \times 60,000(\mathrm{Da}) \quad (5)$$

$$V = 70.579(\mathrm{nm}^3) \quad (6)$$

where V is the volume of the protein, p is the density of the protein (in grams/cubic centimeter), M is the mass of the protein (in daltons), and Na is Avogadro constant.

Assuming a sphere with volume V, the diameter (d) is calculated as follows:

$$d(\mathrm{nm}) = 2 \times (3V/4\pi^{1/3}) \quad (7)$$

$$d(\mathrm{nm}) = 2 \times [3 \times 70.579(\mathrm{nm}^3)/4\pi^{1/3}] \quad (8)$$

$$d = 2.563\ \mathrm{nm} \quad (9)$$

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